TIRC Grant #203

Reprinted from JOURNAL OF CHRONIC DISEASES, St. Louis
Vol. 8, No. 6, Pages 661-668, December, 1958 (Printed in the U. S. A.)

FURTHER STUDIES ON CHOLESTEROL LEVELS IN THE JOHNS HOPKINS MEDICAL STUDENTS: THE EFFECT OF STRESS AT EXAMINATIONS

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(Received for publication Sept. 5, 1958)

THE variability of serum cholesterol levels found in healthy subjects on repeated determinations depends on many factors.[1,2] When the method has been carefully standardized and the technical error of measurement is small, there are still wide variations in the cholesterol level of a given individual, occurring both from week to week and from year to year, which cannot be attributed entirely to age or to diet.[1,3] The influence of various forms of stress on the cholesterol level is of increasing interest and importance. A number of investigators have found that cholesterol levels are appreciably higher during periods of stress than at other times.[4-9] While the exact significance of this finding is not yet clear, the possibility exists that such elevations of cholesterol level may contribute to the early onset of coronary artery disease in some persons.

As part of a long-term study on possible precursors of hypertension and coronary disease, we have made continuing studies of the cholesterol levels of successive classes of the Johns Hopkins medical students. The class of 1961, entering in the fall of 1957, was the fourteenth class registered in the study. It was decided to obtain cholesterol determinations on that class during the final anatomy examination period to compare with other levels obtained during the

This study was supported in part by Research Grant H-1891, National Heart Institute, and in part by the Tobacco Industry Research Committee.

same year, since it is generally considered that the final anatomy examination is a severe form of stress, probably the greatest encountered by the medical students as a whole during their four academic years.

METHOD

The class of 1961 was examined in the different branches of anatomy over a period of 5 days in January, 1958. Each student had oral examinations on 3 successive days followed by 2 days of practical examinations. Through the kind cooperation of Dr. David Bodian, Professor of Anatomy, the students were asked to come to the laboratory for stress studies immediately after one of these examinations. Body weight, blood pressure, and heart rate were measured, then blood was drawn for a blind duplicate cholesterol determination and a circulating eosinophil count. Data on the same subjects were also obtained at the entrance physical examination carried out in the autumn by members of the staff of the Department of Medicine and also when, at a varying interval after the anatomy examination period, the subject came to our laboratory for research purposes by individual appointment. Total serum cholesterol was determined in the Clinical Chemistry Laboratory of The Johns Hopkins Hospital by the Buell modification of the Bloor method as previously described.[1] Total circulating eosinophils were counted by the method of Hills, Forsham, and Finch.[10]

The three tests are identified in chronologic order by Roman numerals. Details concerning the collection of data at each test are shown in Table I. Although there were minor differences in the frames of reference, none are of much importance except that blood pressure and heart rate were measured sitting in Test II and recumbent in Tests I and III. Time of day was similar for Tests II and III, so that the eosinophil counts are quite comparable in that respect.

TABLE I

TEST	OCCASION	DATE	WEIGHT	BLOOD PRESSURE AND HEART RATE	NUMBER OF SERUM CHOLESTEROL SAMPLES	EOSINOPHIL COUNT
I	Admission physical examination	Oct. 1-3, 1957	In shirt, trousers, and shoes	Initial, recumbent	1	None obtained
II	Anatomy examination	Jan. 6-10, 1958	Same as I	Initial, sitting	2	Direct count
III	Laboratory studies	Jan. 21-April 24, 1958	In trousers and shoes	Initial, recumbent	2	Direct count

For the sake of consistency, the statistical analysis in this report is based on the protocols of the 52 men with complete cholesterol data. The 17 remaining men in the class of 1961 were not included for the reasons shown in Table II. The 5 women were excluded because of reports of fluctuation of cholesterol level with the menstrual cycle, which would introduce another biologic variable.[11,12]

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TABLE II

REASON	NUMBER
Did not report for Test II	11*
Originally in the class of 1960; had Test III the previous year	2
One determination only at Test II	2
One determination only at Test III	2

*Two of these men had stress studies at the time of the final biochemistry examination; their findings are presented separately, but are not included in the group data.

The 52 men studied were 19 through 26 years of age; three were 19 to 20, four were 24 to 26, and the rest were 21 to 23 years old. All were in good health. None had hypertension, coronary disease, diabetes, nephrosis, thyroid disease, or any other disorder thought to influence cholesterol level. There were no pronounced variations in habits of exercise, diet, or smoking between Tests II and III. For many subjects, exercise habits and diet were undoubtedly different during the summer than after entering medical school, so that the conditions preceding Test I, carried out 16 to 18 days after admission, were less consistent in these respects. These studies could not be directly controlled as to possible seasonal variations in cholesterol level in the absence of stress, since all medical students undergo stressful situations periodically. A study of seasonal cholesterol values among institutionalized patients or prisoners in this locality might provide helpful information in this regard.

RESULTS

The cholesterol levels of the 52 male medical students at final anatomy examination and at two other times of year are shown in Table III. It will be seen that the highest mean cholesterol value (225.7 mg. per 100 c.c.) was present at the time of the anatomy examination and the mean level of 204.7 mg. per 100 c.c. at Test III was significantly lower ($P < 0.001$). However, there was no significant difference between the mean level of 224.4 mg. per 100 c.c. at Test I, carried out 16 to 18 days after entering medical school, and that of Test II. Following Test II, 39 subjects exhibited a fall of 67.5 to 7.5 mg. per 100 c.c. in cholesterol level, averaging 31.4 mg. per 100 c.c.; four showed no change, and nine subjects showed a rise of 4.0 to 28.5 mg. per 100 c.c., averaging 14.8 mg. per 100 c.c. It is noteworthy that two-thirds of the group showing a rise had cholesterol levels under 200 mg. per 100 c.c. both at Test II and Test III, while less than one-quarter of those with a fall in cholesterol after Test II showed a similar low range at both tests. Two subjects had hypercholesteremic levels* at Test II but not at Test III. Hypercholesteremia was also present at Test I in Subject 61114 but not in Subject 61156. In these two subjects, the fall in cholesterol between Tests II and III of 40 and 67 mg. per 100 c.c., respectively, is somewhat greater than the mean for the group of 39 as a whole.

*Cholesterol of 300 mg. per 100 c.c. or more.

TABLE III. CHOLESTEROL LEVELS OF 52 MALE MEDICAL STUDENTS AT ANATOMY EXAMINATION COMPARED WITH TWO OTHER TIMES OF YEAR

		CHOLESTEROL LEVEL (MG. PER 100 C.C.)					
		I	II	III	II-I	III-II	III-I
Mean		224.4	225.7	204.7	1.3	−21.0	−19.6
S.E. mean		±5.396	±5.539	±4.311	±4.394	±3.367	±3.835
Median		221.5	229.0	202.2	2.2	−21.0	19.0
t					0.302	6.225****	5.120****
Direction of change	+				27	9	12
	0				0	4	0
	−				25	39	40

I = At admission physical examination Oct. 1-3, 1957.
II = At final anatomy examination Jan. 6-10, 1958.
III = At our laboratory later in 1958 by individual appointment.
****P <0.001.

TABLE IV

SUBJECT NUMBER	AGE	SAMPLE NUMBER	CHOLESTEROL (MG. PER 100 C.C.)		
			TEST I	TEST II	TEST III
61114	23	1	325	305	270
		2	—	315	270
		Mean	—	310	270
61156	23	1	287	305	248
		2	—	325	248
		Mean	—	315	248

Since the mean cholesterol level for the entire group fell significantly between Tests II and III, the data were examined to determine whether or not a positive association existed between the degree of lowering of cholesterol and the interval of time between Tests II and III. Test III was carried out in random sequence on the 52 subjects 13 to 105 days after Test II. Both the interval between the two tests and the fall in cholesterol between Tests II and III had fairly normal distributions.

The over-all correlation coefficient between fall in cholesterol and interval between Tests II and III was not found to be significant ($r = 0.049$). However, when these two variables were plotted in a scatter diagram, the shadow of the effect of the final biochemistry examination appears to be imprinted there (Fig. 1). The final biochemistry examination fell on March 19, 68 days after the end of the anatomy examination period. For the 16 days prior to the biochemistry examination, no fall in cholesterol level as great as 25 mg. per 100 c.c. was found,

and not until 10 days after that examination were such falls again present. It is also noteworthy that no fall of great magnitude was observed in the first 3 weeks after the anatomy examination.

The cholesterol levels of the two subjects who had stress studies at the biochemistry examination rather than at the anatomy examination are pertinent here.* Their data are not included in Table III or Fig. 1. Chronologically the time relationship of the biochemistry stress studies to Test III was just the opposite of the anatomy stress studies, that is, the stress studies followed Test III rather than preceded it. The change in cholesterol level paralleled this reversal. Rises of 30 and 25 mg. per 100 c.c., respectively, were recorded between Test III and the biochemistry examination. Thus it appears that higher cholesterol values were associated with both the anatomy and the biochemistry examinations and lower cholesterol values were more frequently found at periods of time beginning 2 or 3 weeks after the examination in question was over.

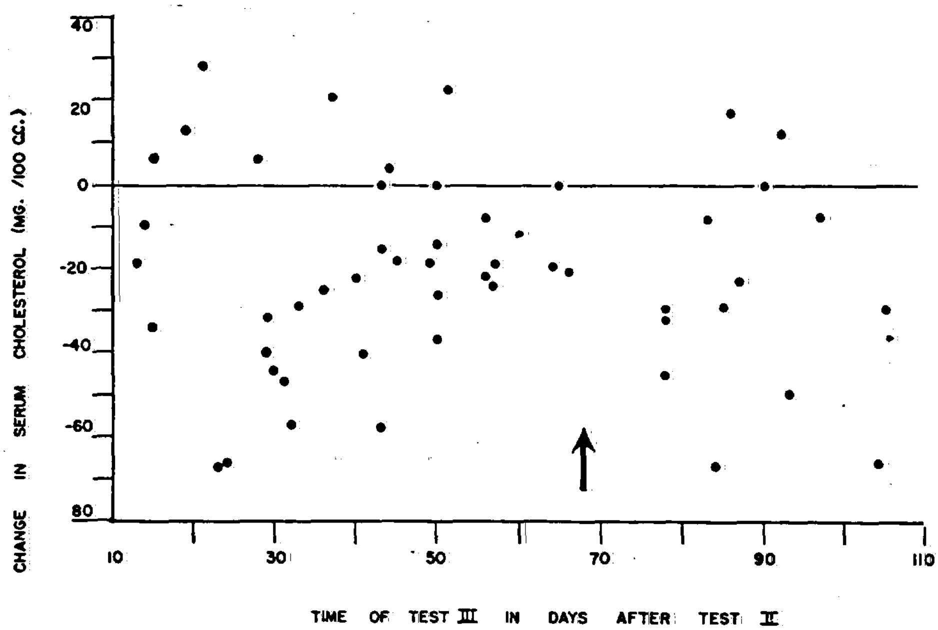

Fig. 1.—The relationship of the change in cholesterol level to the interval between the anatomy examination (Test II) and Test III among 52 Johns Hopkins students. The change in cholesterol level expressed in milligrams per 100 c.c. Each dot represents the absolute change for a single subject. Test II was carried out at final anatomy examination. Test III was performed in our laboratory by individual appointment for research purposes only 13 to 105 days after Test II.

† Indicates March 19, 1958, the day of the final biochemistry examination.

That the change in cholesterol level between Tests II and III did not depend on change in body weight is demonstrated in Table VI. Mean body weight decreased about one pound between Tests I and II while cholesterol remained stable but did not change between Tests II and III while cholesterol decreased. The small weight change between Tests I and II was not statistically significant.

*These two men, the only ones of the 11 absentees at Test II who came on request for Test IV, happened to have astonishingly similar data.

TABLE V

SUBJECT	SAMPLE	TEST I	TEST II (ANATOMY)	TEST III	TEST IV (BIOCHEMISTRY)
61104		Oct. 3, 1957	Jan. 6-10, 1958	Jan. 23, 1958	March 19, 1958
	1	200	—	165	200
	2	—	—	180	205
	Mean	—	—	172.5	202.5
61136		Oct. 1, 1957	—	Feb. 27, 1958	March 19, 1958
	1	200	—	180	200
	2	—	—	175	205
	Mean	—	—	177.5	202.5

TABLE VI. BODY WEIGHT, CIRCULATING EOSINOPHILS, BLOOD PRESSURE, AND HEART RATE AT ANATOMY EXAMINATION (II) COMPARED WITH TWO OTHER TIMES OF YEAR

MEASUREMENT	N		I	II	III	II-I	III-II	III-I
Body weight	52	Mean	172.1	171.0	171.0	−1.1	0.0	−1.2
		S.E. mean	±2.93	±3.19	±3.11	0.74	±0.25	±0.74
		t	—	—	—	1.52	0.08	1.58
Total circulating eosinophils (c.mm.)	47	Mean	—	97.0	129.1	—	32.1	—
		S.E. mean	—	±9.45	14.53	—	±11.06	—
		t	—	—	—	—	2.90**	—
Systolic pressure	52	Mean	122.4	128.7	122.7	6.2	−5.9	0.3
		S.E. mean	±1.63	±1.49	±1.22	±2.05	±1.88	±1.83
		t	—	—	—	3.04***	3.16***	0.16
Diastolic pressure	52	Mean	72.5	78.7	64.1	6.2	−14.7	−8.5
		S.E. mean	±1.23	±1.42	±1.21	±1.76	±1.20	±1.53
		t	—	—	—	3.53****	12.19****	5.52****
Heart rate	52	Mean	77.4	81.0	75.0	3.6	−6.0	−2.4
		S.E. mean	±1.57	±1.97	±1.61	±1.86	±2.05	±1.51
		t	—	—	—	1.92	2.94***	1.61

** $0.01 > P > 0.005$.
*** = $0.005 > P > 0.001$.
**** = $P < 0.001$.

It is now well known that the number of circulating eosinophils in the peripheral blood falls during periods of stress of various kinds. Although eosinophil counts were not obtained at Test I, 47 of the 52 subjects under investigation had eosinophil counts both at Test II and Test III. Their mean eosinophil count at Test II, the anatomy examination, was 97 per c.mm., while at Test III it was 129 per c.mm. Assuming that the more usual values were present at

[redacted]

Test III, a significant fall of 32 per c.mm., or nearly 25 per cent of the total circulating eosinophils, appears to have taken place at the time of the anatomy examination, with subsequent recovery.

In regard to the findings concerning blood pressure and heart rate, it should be recalled first that the subjects were sitting at Test II and recumbent at Tests I and III. With this distinction in mind, the significant differences indicated in Table IV, all but one of which involve comparison of Tests I or III with Test II, must be interpreted with caution. The finding that the systolic pressure at Test II is significantly higher than at Tests I or III, when the mean levels were almost identical, could result from the effect of posture alone without regard to stress. Both diastolic pressure and heart rate show a gradation in mean values, with the highest level at Test II and the lowest at Test III; Test I assumes an intermediate position. The three means are significantly different from each other for diastolic pressure only, but a similar trend exists in regard to heart rate. Although too much emphasis should not be placed on the elevations of diastolic pressure and heart rate at Tests II and I, they suggest that stress as well as posture may be playing a role.

DISCUSSION

We have presented statistical evidence showing that the mean cholesterol level was higher at the anatomy examination than at a later, less stressful time. This finding is consistent with the hypothesis that serum cholesterol levels rise during periods of stress. Moreover, the eosinophil data supports the view that the students as a group were actually experiencing more stress at Test II than at Test III. If, as seems most likely, the elevation of cholesterol encountered at Test II was indeed due to stress, then the similar elevation at Test I was probably the result of stress from a somewhat different cause. At Test I, the students were going through an intensive period of adaptation, having been in medical school less than 3 weeks. They were dissecting a human cadaver for the first time, in addition to becoming accustomed to new living conditions, new classmates, and new methods of teaching. This unexpected finding added to some evidence that the biochemistry examination was also accompanied by elevation of cholesterol indicates that situations stressful enough to make a significant difference in cholesterol level are not uncommon, at least in a medical student's life.

These findings are similar to those of Wertlake and his colleagues,[9] whose report on 44 medical students appeared while this study was in progress. They found an 11 per cent rise in cholesterol from the control period to the stress period. Using Test III as our control period, our subjects showed a 10.3 per cent elevation of cholesterol at Test II and a 9.6 per cent elevation at Test I. The mechanism of this change, if indeed it be due to stress, remains to be explained.

SUMMARY

1. Serum cholesterol determinations on 52 male medical students were carried out at admission to medical school (Test I), at final anatomy examination (Test II), and during a period of regular academic work (Test III).

2. The mean cholesterol levels at Tests I and II were significantly higher than at Test III.

3. The cholesterol levels of two additional subjects showed a similar pattern when the values at final biochemistry examination (Text IV) were compared with Test III.

4. For certain students, Test III fell close to the final biochemistry examination; the fall from Test II to Test III was less marked in these subjects.

5. The total eosinophil count was significantly lower at Test II than Test III.

6. There was no significant difference in body weight between Tests I, II, and III.

7. The findings are consistent with the hypothesis that stress such as accompanies the first few weeks of medical school or important final examinations is accompanied by a significant mean rise in cholesterol level.

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